



# General Physics



# 1. Molecule Microscopy

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## 1.1 Scanning Desorption Molecule Microscopy (SDMM)

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Over the last few decades in biology and materials science, many new and valuable probes of bulk and surface properties have evolved. Surface techniques have become especially significant as the importance of the surface has been understood, theoretical developments have taken place, and most important (although at a technical level), as the vacuum in which the sample is placed has been improved to  $10^{-12}$  Torr and better, with monolayer times of  $\sim 10^7$  seconds. The variety of microprobes using electrons, ions, and x-rays for both stimulation and detection has also multiplied greatly, and many of them are being used in the biological sciences, where it is desired to determine, element by element, what is present in a small sample of tissue or other bio-material. The classical imaging instruments, the optical microscope with refinements, interference techniques, television imaging, computer-assisted methods and the electron microscope in its many forms, have all undergone continued development and improvement.

An optical or electron microscope produces an enlarged image with contrasting light and dark regions that reveal differences in the way the sample transmits or reflects light or electrons. In the SDMM, contrast comes from differences in the number of molecules that evaporate (desorb) when different small parts of the surface of the sample are heated (or otherwise stimulated), one after the other. These molecules can have been either part of the sample, or have been applied beforehand as a stain, or can come through the sample. In the case of a stain, the number of molecules evaporated from a part of the sample at different temperatures will reveal the strength of binding of molecules to the surface, thus making it possible, in the case of water, for example, to visualize directly hydrophobic regions where water binds weakly, and hydrophilic regions where water binds strongly. The SDMM in its simplest form produces an image by heating each small part of the sample in turn and brightening the spot of a Cathode Ray Tube (CRT) in proportion to the number of molecules that have evaporated. The spot moves so that it is always in the same relative position on the CRT screen as the part of the sample being heated, and the magnification is therefore approximately the ratio of

the screen-to-sample dimensions.

Why is SDMM potentially and uniquely valuable in biology? Because, to cite only a few applications:

1. It can identify directly the composition of surface components visualized in freeze-fracture by their adsorption and absorption properties for water and other molecules.
2. It can elucidate the nature of sites (often shown schematically by interlocking shapes) through studies of the adsorption of various small molecules on the surfaces of both partners that bind specifically.
3. It can be used to "read" electrophoretic separations directly on a microscopic scale.

Work on the SDMM can be conveniently classified under the following nine headings.

1. A vacuum system with adequate performance.
2. A vacuum lock for easy introduction and removal of samples.
3. Methods for maintaining the sample at a desired temperature and for evaporating molecules from it in a localized way.
4. Methods for applying staining molecules to the sample.
5. Methods for etching away undesired surface layers of the sample in a controlled way.
6. Devices for detecting and selecting species of molecules that evaporate from the sample.
7. Apparatus for processing the resulting signals and for forming images.
8. Suitable test samples, and studies in collaboration with interested biologists.
9. A catalog of staining molecules and their behavior relative to various substrates of biological interest with various degrees of denaturation.

Since each part interacts with the others, we have given varying amounts of attention to each as dictated by the needs of the moment. Progress in 1981 was principally under headings 3, 6, and 8, as follows:

A new method of localized heating was adopted in which focused pulses of laser light are used. The sample is placed in vacuum on the surface of a 50-nm thick layer of evaporated tellurium supported by a 2-mm thick plexiglass disk. Light from a 12-mw He-Ne laser passes through an acousto-optical modulator and is focused on a 4  $\mu\text{m}$  optical fiber which brings the light to a lens system that can be mechanically scanned across the tellurium film. It was found readily possible to melt 5  $\mu\text{m}$  holes in the tellurium film with 50  $\mu\text{m}$  pulses.

The instrument's ability to distinguish spatial variations in adsorbate binding density was demonstrated by thermally desorbing labeled water ( $\text{HD}^{18}\text{O}$ ) from a model biological surface with  $5\text{ }\mu\text{m}$  spatial resolution. The test sample consisted of a film of stearic acid on the metallic film of the laser desorption apparatus; water binds to the stearic acid but does not bind to the metal.

Pictures were thus obtained of the edge of the stearic acid spot, and of squamous (cheek) cells. Because the scanning was both mechanical and by hand, the pictures were crude, but they show that all the necessary elements work. With better optics to give  $1\text{ }\mu\text{m}$  spots, higher detection efficiency, and automatic scanning, we can expect useful performance from the SDMM.

Considerable effort has been put into designing more efficient neutral molecule detectors. Because the laser desorption is very rapid, it produces bursts of molecules in vacuum of small extent, and it makes sense to ionize them with intense pulses of electrons or light, and to use time-of-flight mass spectrometry. The simplest scheme is to increase the solid angle by using a low-current sheet of electrons running parallel to the sample. Next, one can use a high-current pulse of electrons; and finally, a 0.2-J, 10-ns pulse of 193-nm light from an Ar-F excimer laser. Improvements in detection efficiency of  $10^2$ ,  $10^4$ , and  $10^6$ , respectively, can be expected.

## 1.2 Scanning Micropipette Molecule Microscopy (SMMM)

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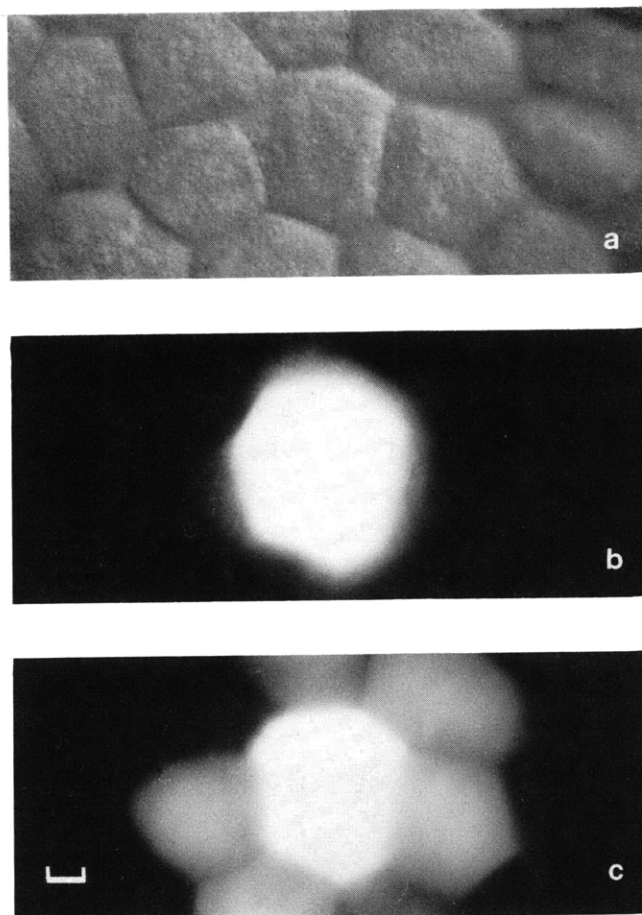
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The major goal of this effort has been to develop an instrument with spatial resolution of  $1\text{ }\mu\text{m}$ , to study in vitro transport across epithelia (biological tissues). Light and electron microscopy have been enormously useful in revealing the structure of biological materials. Radioisotopes and other techniques have provided information about average transport rates across living tissues. SMMM provides a link between our knowledge of transport and our knowledge of structure by allowing direct mapping of transport onto microscopic structure.

The SMMM has a perfusion chamber that allows simultaneous observation of the sample with a light microscope and the introduction into the field of view of a glass micropipette with a tip diameter of  $1\text{--}10\text{ }\mu\text{m}$ . The tip of the micropipette is sealed with a small plug of permeable material such as dimethyl silicone rubber or cellulose acetate. The inside of the micropipette is connected via a flexible vacuum coupling to the inlet of the ionizer of a quadrupole mass spectrometer; hence it is under vacuum. The micropipette is inserted in solution and scanned over (or placed at various points on) the surface of the sample. Whatever molecules present that can permeate the plug in sufficient



**Figure 1-1:** Sequential view of reversible uncoupling experiment. (a) Interference contrast view (40X water-immersion objective) of apical surface of *Necturus* gall bladder before impalement. (b) Epifluorescent view of injected cell in uncoupled epithelium (80% CO<sub>2</sub>, 32 mM bicarbonate buffer) 10 minutes after injection with the fluorescent dye, Lucifer Yellow. No dye spreading is visible. (c) Epifluorescent view of epithelium showing dye coupling after switching to air-saturated buffer. Scale bar, 10  $\mu$ m.

numbers are detected by the quadrupole mass spectrometer. Thus spatial variations in the concentrations of the permeating molecules can be mapped out and correlated with the *in vitro* morphology as observed with the light microscope. A detailed description has been published elsewhere.<sup>1,2</sup>

During the past year, our work has focused on the continued improvement of the sensitivity and resolution of the SMMM and the development of techniques for microinjection of fluorescent dyes

into both native and cultured epithelial cells. The latter techniques will enable us to study, using the SMMM, the role of intercellular communication in the hormonal control of water reabsorption in tissues that model the distal tubules of the mammalian kidney.

We are also using these microinjection techniques to study the development of intercellular communication between cultured cells, in collaboration with Dr. Carlos Rabito at Massachusetts General Hospital. Epithelial cells in culture start out in suspension as individual rounded cells. These cells are allowed to settle down on microscope cover slips. The cells then start to spread out and divide until they form a confluent monolayer, at which point cell growth and division stop. The question we are trying to answer is: At what point during this growth phase do the cells establish intercellular communication, and is this the means by which growth is terminated? This is an important process in development. It has even been suggested that the inability to form gap junctions and establish intercellular communication is an important characteristic of tumor cells, i.e., the signal to stop growing can never be given, thus allowing uncontrolled growth.

Derivative from this work, we have also been investigating which agents can modulate intercellular communication. In particular, we have recently shown that dye coupling in *Necturus* gall bladder (a marker for intercellular communication) is reversibly inhibited by high concentration of dissolved carbon dioxide, which acts to lower intracellular pH (Figure 1-1). This contrasts with recently published work<sup>3</sup> in which it was shown that the intercellular communication between mature chick embryo lens cells is resistant to carbon-dioxide-induced uncoupling and is consistent with the hypothesis that the properties of gap junctions differ in different epithelia.

### References

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